

WHAT IS CLAIMED IS:

1. A method for producing an enzymatically active lysosomal enzyme or modified lysosomal enzyme in a transgenic plant, comprising:

- Sub A'
- 5 (a) growing the transgenic plant which has a recombinant expression construct comprising a nucleotide sequence encoding the lysosomal enzyme or modified lysosomal enzyme and a promoter that regulates expression of the nucleotide sequence so
- 10 that the lysosomal enzyme or modified lysosomal enzyme is expressed by the transgenic plant; and
- (b) recovering the lysosomal enzyme or modified lysosomal enzyme from an organ of the transgenic plant;
- 15 wherein the modified lysosomal enzyme has the amino acid sequence of the lysosomal enzyme with one or several amino acid substitutions, additions and/or deletions, and the organ is a leaf, stem, root, flower, fruit or seed.

- 20 2. The method according to claim 1, in which the promoter is an inducible promoter, and which method additionally comprises, between steps (a) and (b), the step of inducing the inducible promoter before or after the transgenic plant is harvested.

- 25 3. The method according to claim 2, in which the inducible promoter is induced by mechanical gene activation.

4. The method according to claim 3, in which the
- 30 inducible promoter comprises SEQ ID NO:5.

- Sub B
5. The method according to claim 1, in which the modified lysosomal enzyme comprises a detectable marker peptide fused to the amino or carboxyl terminal of the
- 35 lysosomal enzyme.

6. The method according to claim 5, 7, in which the detectable marker peptide comprises SEQ ID NO:10.

7. The method according to claim 1, in which the transgenic plant is a transgenic tobacco plant.

8. The method according to any of claims 1, 4 and 7, in which the lysosomal enzyme or modified lysosomal enzyme is a human lysosomal enzyme or modified human lysosomal enzyme.

9. The method according to claim 8, in which the human lysosomal enzyme or modified human lysosomal enzyme is a glucocerebrosidase, modified glucocerebrosidase, α -L-iduronidase or modified α -L-iduronidase.

10. A recombinant expression construct comprising a nucleotide sequence encoding a lysosomal enzyme or modified lysosomal enzyme and a promoter that regulates the expression of the nucleotide sequence in a plant cell, wherein the modified lysosomal enzyme has the amino acid sequence of the lysosomal enzyme with one or more amino acid substitutions, additions and/or deletions.

11. The recombinant expression construct of claim 10, in which the promoter is an inducible promoter.

12. The recombinant expression construct of claim 11, in which the inducible promoter is induced by mechanical gene activation.

13. The recombinant expression construct of claim 12, in which the inducible promoter comprises SEQ ID NO:5.

14. The recombinant expression construct of claim 10, in which the modified lysosomal enzyme comprises a detectable

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marker peptide fused to the amino or carboxyl terminal of the lysosomal enzyme.

15. The recombinant expression construct of claim 14, 24
5 in which the detectable marker peptide comprises SEQ ID
NO:10.

16. The recombinant expression construct of claim 10 or
13, in which the lysosomal enzyme or modified lysosomal
10 enzyme is a human lysosomal enzyme or modified human
lysosomal enzyme.

17. The recombinant expression construct of claim 16,
in which the human lysosomal enzyme or modified human
15 lysosomal enzyme is a glucocerebrosidase, modified
glucocerebrosidase, α -L-iduronidase or modified α -L-
iduronidase.

18. A plant transformation vector comprising the
20 recombinant expression construct of claim 16.

a² 19. A plant transformation vector comprising the
recombinant expression construct of claim 17.

25 20. A plant cell, tissue or organ which has the
recombinant expression construct of claim 16.

a³ 21. A plant cell, tissue or organ which has the
recombinant expression construct of claim 17.

30 22. A plasmid CTPro1:hGC:FLAG having the ATCC accession
number 97277.

23. A plasmid pCT22 having the ATCC accession number
35 97701.

24. A plasmid pCT54 having the ATCC accession number

5 25. A transgenic plant or plant cell capable of producing an enzymatically active lysosomal enzyme or modified lysosomal enzyme, which transgenic plant or plant cell has a recombinant expression construct comprising a nucleotide sequence encoding a lysosomal enzyme or modified
10 lysosomal enzyme and a promoter that regulates expression of the nucleotide sequence in the transgenic plant or plant cell, wherein the modified lysosomal enzyme has the amino acid sequence of the lysosomal enzyme with one or more amino acid substitutions, additions and/or deletions.

15 26. The transgenic plant or plant cell of claim ⁴¹25, in which the promoter is an inducible promoter.

20 27. The transgenic plant or plant cell of claim ⁴²26, in which the inducible promoter is induced by mechanical gene activation.

25 28. The transgenic plant or plant cell of claim ⁴³27, in which the inducible promoter comprises SEQ ID NO:5.

29. The transgenic plant or plant cell of claim 25, in which the modified lysosomal enzyme comprises a detectable marker peptide fused to the amino or carboxyl terminal of the lysosomal enzyme.

30 30. The transgenic plant or plant cell of claim ⁴⁶29, in which the detectable marker peptide comprises SEQ ID NO:10.

35 31. The transgenic plant or plant cell of claim ⁴⁰30, in which the transgenic plant or plant cell is a transgenic tobacco plant or tobacco cell.

32. The transgenic plant or plant cell of any of claims 25, 28 and 31, in which the lysosomal enzyme or modified lysosomal enzyme is a human lysosomal enzyme or modified human lysosomal enzyme.

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33. The transgenic plant or plant cell of claim 32, in which the human lysosomal enzyme or modified human lysosomal enzyme is a glucocerebrosidase, modified glucocerebrosidase, α -L-iduronidase or modified α -L-iduronidase.

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34. A leaf, stem, root, flower or seed of the transgenic plant of claim 32.

35. A leaf, stem, root, flower or seed of the transgenic plant of claim 33.

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36. A plant grown from a seed of plant line X-11, which seed has the ATCC Accession No. 97275.

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37. A plant grown from a seed of plant line X-27, which seed has the ATCC Accession No. 97276.

38. A plant grown from a seed of plant line CT40-9, which seed has the ATCC Accession No. 97700.

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39. A lysosomal enzyme or modified lysosomal enzyme which is enzymatically active and is produced according to a process comprising:

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- (a) growing a transgenic plant which has a recombinant expression construct comprising a nucleotide sequence encoding the lysosomal enzyme or modified lysosomal enzyme and a promoter that regulates expression of the nucleotide sequence so that the lysosomal enzyme or modified lysosomal enzyme is expressed by the transgenic plant; and

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(b) recovering the lysosomal enzyme or modified lysosomal enzyme from an organ of the transgenic plant;

wherein the modified lysosomal enzyme has the amino acid
5 sequence of the lysosomal enzyme with one or more amino acid substitutions, additions and/or deletions, and the organ is a leaf, stem, root, flower, fruit or seed.

40. The lysosomal enzyme or modified lysosomal enzyme
10 of claim 39, in which the promoter is an inducible promoter, and which process additionally comprises, between steps (a) and (b), the step of inducing the inducible promoter before or after the transgenic plant is harvested.

15 41. The lysosomal enzyme or modified lysosomal enzyme of claim 40, in which the inducible promoter comprises SEQ ID NO:5.

42. The lysosomal enzyme or modified lysosomal enzyme
20 of claim 39, in which the modified lysosomal enzyme comprises a detectable marker peptide fused to the amino or carboxyl terminal of the lysosomal enzyme.

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43. The lysosomal enzyme or modified lysosomal enzyme
25 of claim 42, in which the detectable marker peptide comprises SEQ ID NO:10.

44. The lysosomal enzyme or modified lysosomal enzyme
of claim 39, in which the transgenic plant is a transgenic
30 tobacco plant.

45. The lysosomal enzyme or modified lysosomal enzyme
of any of claims 39, 41 and 44, in which the lysosomal enzyme or modified lysosomal enzyme is a human lysosomal enzyme or
35 modified human lysosomal enzyme.

46. The lysosomal enzyme or modified lysosomal enzyme
of claim 45, in which the human lysosomal enzyme or modified
human lysosomal enzyme is a glucocerebrosidase or modified
glucocerebrosidase, α -L-iduronidase or modified α -L-
5 iduronidase.

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